IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: James P. Elia )
)
SERIAL NO.: 09/836,750 ) EXAMINER: Elizabeth C. Kemmerer
)
FILED: April 17, 2001 )
) GROUP ART UNIT: 1646
FOR: METHOD FOR GROWING )
MUSCLE IN A HUMAN HEART )



**THIRD SUPPLEMENTAL DECLARATION**
**OF ANDREW E. LORINCZ, M.D.**

I, Andrew E. Lorincz, declare as follows:

1. I reside at 16135 NW 243rd Way, High Springs, Florida 32643-3813.

2. This Third Supplemental Declaration is submitted in addition to my previous Declaration, dated June 9, 2003, my Supplemental Declaration dated February 3, 2004, and my Second Supplemental Declaration dated July 19, 2004. No changes are made to any of such previous Declarations.

3. My Curriculum Vitae (hereinafter "CV") is attached as Exhibit A to my Declaration of June 9, 2003, and my background is further amplified by materials submitted in my Second Supplemental Declaration.

4. I have read and understood the disclosures of the above-referenced patent application at page 20, line 10 through page 21, line 15; and page 44, line 19 through page 46, line 16. Such disclosures are the same as I read and understood

in my previous Declaration and Supplemental Declaration. A copy of such disclosures is attached hereto as Third Supplemental Declaration Exhibit A.

I have also read and understood additional disclosures of the above-referenced patent application at page 33, lines 8-10; page 37, lines 19-25; page 40, line 20 through page 43 line 3; page 44, lines 12 and 13; page 48, lines 13-15; page 53, line 1 through page 56, line 25; and page 62, lines 1-10. A copy of such additional disclosures is attached hereto as Third Supplemental Declaration Exhibit B.

5. The disclosures in Third Supplemental Declaration Exhibit A, also contained in my previous Declaration and Supplemental Declaration, relate to using growth factors, including cells, for promoting the growth of soft tissue and, more specifically, to a method which may use such growth factors for growing a new portion of a human heart by growing new cardiac muscle. Such disclosures are also directed to the growth of new arteries in the heart.

I understand that the additional disclosures in Third Supplemental Declaration Exhibit B relate to using cellular growth factors, including bone marrow stem cells, to grow soft tissue, including an artery. Stem cells harvested from bone marrow, peripheral blood and from culture banks are described as being implanted for promoting morphogenesis and growth of all three-germ tissue layers, i.e. mesoderm, ectoderm and endoderm tissues. It would be understood by one skilled in the art that morphogenesis includes the growth of an artery, which comprises mesodermal tissue.

6. I have read and understood the claims set forth in Third Supplemental Declaration Exhibit C and have been informed that such claims will be concurrently presented in this application with this Third Supplemental Declaration.

7. Based upon above Paragraphs 4-6, it is my opinion that one skilled in the medical arts, armed with the knowledge in the disclosures referenced therein, would be enabled to practice the method set forth in Third Supplemental Declaration Exhibit C and to predictably anticipate the results defined therein without need for resorting to undue experimentation. It is my further opinion that one skilled in the art reading such disclosures would understand that all of the well known administration procedures described at page 45 of the patent application, including intravenous, intraluminal, intramuscular, and with an angioplasty balloon, would be applicable for use in growing an artery in a human patient regardless of whether the genetic material was a gene; cell, including stem cells such as bone marrow stem cells; or another type of growth factor.

Declarant states that the above opinion was reached independently.

Declarant understands that (1) any willful false statements and the like made herein are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon, and (2) that all statements made of Declarant's own knowledge are true and that all statements made on information and belief are believed to be true.

Further Declarant sayeth not.

Date: 5 June 2006

Andrew E. Lorincz, M.D.

# THIRD SUPPLEMENTAL DECLARATION

# EXHIBIT A

# DISCLOSURES

# EXHIBIT A

# DISCLOSURES
# APPLICATION SERIAL NO. 09/836,750

**PAGE 20, LINE 10 – PAGE 21, LINE 15**

Growth factors can be utilized to induce the growth of "hard tissue" or bone and "soft tissues" like ectodermal and mesodermal tissues. As used herein, the term growth factor encompasses compositions and living organisms which promote the growth of hard tissue, such as bone, or soft tissue, in the body of a patient. The compositions include organic and inorganic matter. The compositions can be genetically produced or manipulated. The living organisms can be bacteria, viruses, or any other living organism which promote tissue growth. By way of example and not limitation, growth factors can include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (acidic/basis (FGF a,b), interleukins (IL's), tumor necrosis factor (TNF), transforming growth factor (TGF-B), colony-stimulating factor (CSF), osteopontin (Eta-1 OPN), platelet-derived growth factor (PDGF), interferon (INF), bone morphogenic protein 1 (BMP-1), and insulin growth factor (IGF). Recombinant and non-recombinant growth factors can be utilized as desired. Bacteria or viruses can, when appropriate, be utilized as growth factors. For example, there is a bacterial hydrophilic polypeptide that self-assembles into a nanometer internal diameter pore to build a selective lipid body. Various enzymes can be utilized for the synthesis of peptides which contain amino acids that control three-dimensional protein structure and growth. Growth factors can be applied in gels or other carriers which regulate the rate of release of the growth factors and help maintain the growth factors and the carrier, at a desired location in the body. Time release capsules, granules, or other carriers containing growth factor can be activated by tissue pH, by enzymes, by ultrasound,

by electricity, by heat, by selected *in vivo* chemicals or by any other selected means to release the growth factor. The carrier can be resorbable or non-resorbable. Or, the growth factor itself can be activated by similar means. Either the carrier or the growth factor can mimic extracellular fluid to control cell growth, migration, and function. The growth factor can be administered orally, systemically, in a carrier, by hypodermic needle, through the respiratory tract, or by any other desired method. The growth factor an also be administered into a capsule or other man-made composition or structure placed in the body. While administration of the growth factor is presently usually localized in the patient's body, circumstances may arise where it is advantageous to distribute a growth factor throughout the patient's body in uniform or non-uniform concentrations. An advantage to growth factors is that they can often, especially when in capsule form or in some other containment system, be inserted to a desired site in the body by simply making a small incision and inserting the growth factor. The making of such small incision comprises minor surgery which an often be accomplished on an out-patient basis. The growth factors can be multifactorial and nonspecific.

**PAGE 44, LINE 19 – PAGE 46, LINE 16**

Genetic material comprising a portion of a gene, a gene, genes, a gene product (i.e., a composition a gene causes to be produced like, for example, an organ-producing growth factor), growth factor, or an ECM (extracellular matrix) can be used in or on the body to grow an organ to tissue. For example, the vascular epithelial growth factor gene (VEGF) or its growth factor equivalent can be inserted into the body to cause an artery to grow. When insertion of a gene, portion of a gene, gene product, growth factor, or ECM *in vivo* or *ex vivo* is referred to herein in connection with any of the implant techniques of the invention, it is understood that a cell

nutrient culture(s), physiological nutrient culture(s), carrier (s), enhancer(s), promoter(s), or any other desired auxiliary component(s) can be inserted with the gene or at the same location as the gene, growth factor, ECM, etc.

An artery is an organ from the circulatory system. An artery can be grown in the heart, legs, or other areas by injecting a gene or other genetic material into muscle at a desired site. Size, vascularity, simplicity of access, ease of exploitation, and any other desired factors can be utilized in selecting a desired site. The gene is one of several known VEGF genes which cause the production of vascular endothelial growth factors. Several VEGF genes which produce vascular endothelial growth factors are believed to exist because nature intends for there to be several pathways (i.e., genes) which enable the production of necessary growth factors. The existence of several pathways is believed important because if one of the genes is damaged or inoperative, other similar genes can still orchestrate the production of necessary growth factors. VEGF genes are used by the body to promote blood vessel growth. VEGF genes are assimilated (taken in) by muscle cells. The genes cause the muscle cells to make a VEGF protein which promotes the growth of new arteries. VEGF proteins can be made in a lab and injected into a patient intravenously, intraluminally, or intramuscularly to promote the growth of an artery. Or, the genes (or other genetic material) can be applied with an angioplasty balloon, with the assistance of a vector, or by any other method.

It is not always desirable to grow a completely new organ. Sometimes growing a portion of an organ is desirable. For example, in some heart attacks or strokes, a portion of the heart or brain remains viable and a portion dies. An injection of a gene to form cardiac muscle and/or an injection of a gene to form an artery can be utilized to revive or replace the dead portion of the heart. The dead portion of the heart may (or may not) be used as a matrix while the new muscles

and vessels grow. Thus, in this example, a partial new organ is grown in a pre-existing organ. A pacemaker may (or may not) be necessary. A second injection of a gene may (or may not) be necessary to stop cardiac muscle growth once it is completed. Portions of organs throughout the body can similarly be repaired or replaced. It may be necessary to provide gene(s) or growth factor(s) sequentially. For instance, one or more blood vessels are grown by inserting an appropriate gene or other genetic material into a selected area. Second, an appropriate gene or other genetic material is inserted in the selected area to grow a bone or other organ.

The size and shape limitation of the desired structure can come from a containment and boundary contact inhibition phenomenon or by a chemical inhibition.

A variation on the theme of growing a portion of an organ is as follows: a portion of a heart dies. The pericardium is utilized as a scaffold and seeded with cells and/or genes to grow new muscle, and genes (or other genetic material) to grow new arteries. Immediately adjacent the dead cardiac muscle, onto or into the pericardium, the appropriate cells, genes, and/or growth factors (or other genetic material) are placed. Once the new muscle and blood vessels have grown, the function specific tissue can be applied to the damaged portion of the heart and paced, if necessary, to augment cardiac action. If the surgeon desires, the dead muscle can be removed and the new muscle and blood vessels can be surgically rotated into the excised region and secured. This probably can be done endoscopically. In essence, the pericardium is utilized to allow the new muscle wall to grow. The new muscle wall is then transplanted into the damaged heart wall. This procedure utilizes the body as a factor to grow an organ and/or tissue, after which the organ and/or tissue is transplanted to a desired region. On the other hand, the new muscle wall may integrate itself into the old wall and not require transplantation.

# THIRD SUPPLEMENTAL DECLARATION

# EXHIBIT B

# DISCLOSURES

# EXHIBIT B

# DISCLOSURES
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**PAGE 33, LINES 8-10**

Morphogenesis or morphogenetics is the origin and evolution of morphological characters and is the growth and differentiation of cells and tissues during development.

**PAGE 37, LINES 19-25**

Multifactorial and nonspecific cells (such as stem cells and germinal cells) can provide the necessary in vivo and in vitro cascade of genetic material once an implanted master control gene's transcription has been activated. Likewise, any host cell, clone cell, cultured cell, or cell would work. Genetic switches (such as the insect hormone ecdysone) can be used to control genes inserted into humans and animals. These gene switches can also be used in cultured cells or other cells. Gene switches govern whether a gene is on or off making possible precise time of gene activity.

**PAGE 40, LINE 20 – PAGE 43, LINE 3**

**EXAMPLE 11**

MSX-1 and MSX-2 are the homeobox genes that control the generation and growth of a tooth. A sample of skin tissue is removed from the patient and the MSX-1 and MXS-2 homeobox gene(s) are removed from skin tissue cells. The genes are stored in an appropriate nutrient culture medium.

BMP-2 and BMP-4 growth factors are obtained by recombinant or natural extraction from bone.

Living stem cells are harvested from the bone marrow, the blood of the patient, or from cell culture techniques. The stem cells are placed in a nutrient culture medium at 98.6 degrees. The temperature of the culture medium can be varied as desired but ordinarily is between 40 to 102 degrees F.

MXS-1 and MXS-2 transcription factors are obtained which will initiate the expression of the MXS-1 and MXS-2 homeobox genes.

The MXS-1 and MXS-2 transcription factors, BMP-2 and BMP-4 bone morphogenic proteins, and MXS-1 and MXS-2 genes are added to the nutrient culture medium along with the living stem cells.

**EXAMPLE 12**

Example 11 is repeated except that the transcription factors bind to a receptor complex in the stem cell nucleus.

**EXAMPLE 13**

Example 11 is repeated except that the MXS-1 and MXS-2 transcription factors are not utilized. The transcription of the MXS-1 and MXS-2 homeobox genes is activated by applying an electric spark to the nutrient culture medium.

**EXAMPLE 14**

Example 13 is repeated except that the stem cells are starved and the transcription of the MXS-1 and MXS-2 homeobox genes is activated by applying an electric spark to the nutrient culture medium.

## EXAMPLE 15

WT-1 and PAX genes are obtained from a sample of skin tissue is removed from the patient. The genes are stored in an appropriate nutrient culture medium. PAX genes produce PAX-2 and other transcription factors.

BMP-7 and other kidney related BMP growth factors are obtained by recombinant or natural extraction from bone.

Living stem cells are harvested from the bone marrow, the blood of the patient, or from cell culture techniques. The stem cells are placed in a nutrient culture medium at 98.6 degrees. The temperature of the culture medium can be varied as desired but ordinarily is between 40 and 102 degrees F.

The WT-1 and PAX genes, and BMP-7 and other kidney BMPS are added to the nutrient culture medium along with the living stem cells.

A primitive kidney germ is produced. The kidney germ is transplanted in the patient's body near a large artery. As the kidney grows, its blood supply will be derived from the artery.

## EXAMPLE 16

The Aniridia gene is obtained from a sample of skin tissue is removed from the patient. The gene(s) is stored in an appropriate nutrient culture medium.

Aniridia transcription factor (activates expression of the Aniridia gene) and growth factors (function to help stem cells differentiate during morphogenesis to form an eye) are obtained.

Living stem cells are harvested from the bone marrow, the blood of the patient, or from cell culture techniques. The stem cells are placed in a nutrient culture medium at 98.6 degrees.

The temperature of the culture medium can be varied as desired but ordinarily is between 40 to 102 degrees F.

The Aniridia transcription factor and growth factors and the Aniridia gene are added to the nutrient culture medium along with the living stem cells.

A primitive eye germ is produced. The kidney germ is transplanted in the patient's body near the optic nerve. As the kidney grows, its blood supply will be derived from nearby arteries.

**EXAMPLE 17**

The Aniridia gene is obtained from a sample of skin tissue is removed from the patient. The gene(s) is stored in an appropriate nutrient culture medium.

Aniridia transcription factor (activates expression of the Aniridia gene) and growth factors (function to help stem cells differentiate during morphogenesis to form an eye) are obtained and added to the nutrient culture medium.

An eye germ develops. A branch of the nearby maxillary artery is translocated to a position adjacent the eye germ to promote the development of the eye germ. The eye germ matures into an eye which receives its blood supply from the maxillary artery.

The term "cell nutrient culture" as used herein can include any or any combination of the following: the extracellular matrix; conventional cell culture nutrients; and/or, a cell nutrient such as a vitamin. As such, the cell nutrient culture can be two-dimensional, three dimensional, or simply a nutrient, and is useful in promoting the processes of cellular dedifferentiation, redifferentiation, differentiation, growth, and development.

**PAGE 44, LINES 12– 13**

An organ, as used herein, consists of two or more kinds of tissues joined into one structure that has a certain task.

**PAGE 48, LINES 13– 15**

In the example above, if germinal cells (and in some cases, stem cells) are utilized a direct differentiation and morphogenesis into an organ can occur in vivo, ex vivo, or in vitro.

**PAGE 53, LINE 1 – PAGE 56, LINE 25**

**EXAMPLE 18**

A 36 year old Caucasian male experiences pain in his left leg. A medical examination reveals a damaged one inch long section of a large artery in his left leg. The examination also reveals that this damaged section of the artery is nearly completely clogged with plaque and that the wall of the artery is weakened. The weakening in the arterial wall makes attempting to clean out the artery risky and also makes it risky to attempt to insert a stent in the artery.

Recombinant cDNA encoded to combine with a cell ribosome to produce the human growth factor VEGF is assembled into a eukaryotic expression plasmid. The recombinant cDNA is from cDNA libraries prepared from HL60 leukemia cells and is known to cause the growth of arteries. The plasmid is maintained at a room temperature of 76 degrees F.

The clones are placed in 1.0 milliliters of a normal saline carrier solution at a room temperature of 76 degrees F to produce an genetic carrier solution. The genetic carrier solution contains about 250 ug of the cDNA clones. A nutrient culture can, if desired, be utilized in conjunction with or in place of the saline carrier. Each clone is identical. If desired, only a

single clone can be inserted in the normal saline carrier solution. The saline carrier solution comprises 0.09% by weight sodium chloride in water. A saline carrier solution is selected because it will not harm the DNA clone.

Two sites are selected for injection of the genetic carrier solution. While the selection of sites can vary as desired, the sites are selected at the lower end (the end nearest the left foot of the patient) of the damaged section of the artery so that the new arterial section grown can, if necessary, be used to take the place of the damaged section of the artery in the event the damaged section is removed.

The first site is on the exterior wall of the artery on one side of the lower end of the damaged section of the artery. A containment system is placed at the first site.

The second site is inside the wall of the artery on the other side of the lower end of the artery.

The genetic carrier solution is heated to a temperature of 98.6 degrees F. 0.25 milliliters of the genetic carrier solution is injected into the containment system at the first site. 0.25 milliliters of the genetic carrier solution is injected at the second site inside the wall of the artery. Care is taken to slowly inject the genetic carrier solution to avoid entry of the solution into the artery such that blood stream will carry away the cDNA in the solution.

After two weeks, an MRI is taken which shows the patient's leg artery. The MRI reveals new growth at the first and second sites.

After four weeks, another MRI is taken which shows the patient's leg artery. The MRI shows that (1) at the first site a new artery is growing adjacent the patient's original leg artery, and (2) at the second site a new section of artery is growing integral with the original artery, i.e., at the second site the new section of artery is lengthening the original artery, much like inserting

a new section of hose in a garden hose concentric with the longitudinal axis of the garden hose lengthens the garden hose.

After about eight to twelve weeks, another MRI is taken which shows that the new artery growing adjacent the patient's original artery has grown to a length of about one inch and has integrated itself at each of its ends with the original artery such that blood flows through the new section of artery. The MRI also shows that the new artery at the second site has grown to a length of one-half inch.

In any of the examples of the practice of the invention included herein, cell nutrient culture can be included with the gene, the growth factor, the extracellular matrix, or the environmental factors.

In any of the examples of the practice of the invention included herein, the concept of gene redundancy can be applied. For example, the Examples 1 to 14 concerning a tooth list the genes MSX-1 and MSX-2. These genes differ by only two base pairs. Either gene alone may be sufficient. A further example of redundancy occurs in growth factors. Looking at the Examples 10 to 14, BMP4 or BMP2 alone may be sufficient. Redundancy can also be utilized in connection with transcription factors, extracellular matrices, environmental factors, cell nutrient cultures, physiological nutrient cultures, vectors, promotors, etc.

One embodiment of the invention inserts genetic material (gene, growth factor, ECM, etc.) into the body to induce the formation of an organ. Similar inducing materials inserted ex vivo into or onto a living cell in an appropriate physiological nurturing environment will also induce the growth of an organ. The VCSEL laser allows early detection in a living cell of a morphogenic change indicating that organ formation has been initiated. With properly timed transplantation, organ growth completes itself.

During the ex vivo application of the invention, a gene and/or growth factor is inserted into a cell or a group of cells; an ECM or environmental factor(s) are placed around and in contact with a cell or group of cells; or, genetic material is inserted into a subunit of a cell to induce organ growth. An example of a subunit of a cell is an enucleated cell or a comparable artificially produced environment. In in vivo or ex vivo embodiments of the invention to induce the growth of an organ, the genes, growth factors, or other genetic material, as well as the environmental factors or cells utilized, can come from any desired source.

## EXAMPLE 19

Genetically produced materials are inserted in the body to cause the body to grow, reproduce, and replace in vivo a clogged artery in the heart. This is an example of site-specific gene expression. A plasmid expression vector containing an enhancer/promoter is utilized to aid in the transfer of the gene into muscle cells. The enhancer is utilized to drive the specific expression of the transcriptional activator. After the enhancer drives the expression of the transcriptional activator, the transcriptional activator transactivates the muscle/artery genes. Saline is used as a carrier. Cardiac muscle can take up naked DNA injected intramuscularly. Injecting plasmid DNA into cardiac (or skeletal) muscle results in expression of the transgene in cardiac myocytes for several weeks or longer.

Readily available off-the-shelf (RAOTS) cDNA clones for recombinant human VEGF165, isolated from cDNA libraries prepared from HL60 leukemia cells, are assembled in a RAOTS expression plasmid utilizing 736 bp CMV promoter/enhancer to drive VEGF expression. Other RAOTS promoters can be utilized to drive VEGF expression for longer periods of time. Other RAOTS recombinant clones of angiogenic growth factors other than VEGF can be utilized, for example, fibroblast growth factor family, endothelial cell growth

factor, etc. Downstream from the VEGF cDNA is an SV40 polyadenylation sequence. These fragments occur in the RAOTS pUC118 vector, which includes an Escherichia coli origin of replication and the Beta lactamase gene for ampicillin resistance.

The RAOTS construct is placed into a RAOTS 3 ml syringe with neutral pH physiologic saline at room temperature (or body temperature of about 37 degrees C). The syringe has a RAOTS 27 gauge needle.

Access to the cardiac muscle is gained by open heart surgery, endoscopic surgery, direction injection of the needle without incision, or by any other desired means. The cardiac muscle immediately adjacent a clogged artery is slowly injected with the RAOTS construct during a five second time period. Injection is slow to avoid leakage through the external covering of muscle cells. About 0.5 ml to 1.0 ml (milliliter) of fluid is injected containing approximately 500 ug phVEGF165 in saline (N=18). The readily available off-the-shelf cDNA clones cause vascular growth which automatically integrates itself with the cardiac muscle. Anatomic evidence of collateral artery formation is observed by the $30^{th}$ day following injection to the RAOTS construct. One end of the artery integrates itself in the heart wall to receive blood from the heart. The other end of the artery branches into increasing smaller blood vessels to distribute blood into the heart muscle. Once the growth of the new artery is completed, the new artery is left in place in the heart wall. Transplantation of the new artery is not required.

Blood flow through the new artery is calculated in a number of ways. For example, Doppler-derived flow can be determined by electromagnetic flowmeters (using for example, a Doppler Flowmeter sold by Parks Medical Electronic of Aloha, Oregon) both in vitro and in vivo. RAOTS external ultrasound gives a semiquantitative analysis of arterial flow. Also, RAOTS angiograms or any other readily available commercial devices can be utilized.

VEGF gene expression can be evaluated by readily available off-the-shelf polymerase chain reaction (PCR) techniques.

If controls are desired, the plasmid pGSVLacZ containing a nuclear targeted Beta-galactosidase sequence coupled to the simian virus 40 early promoter can be used. To evaluate efficiency, a promoter-matched reporter plasmid, pCMV Beta (available from Clontech of Palo Alto, California), which encodes Beta-galactosidase under control of CMV promoter/enhancer can be utilized. Other RAOTS products can be utilized if desired.

## EXAMPLE 20

A patient, a forty year old African-American female in good health, has been missing tooth number 24 for ten years. The space in her mouth in which her number 24 tooth originally resided is empty. All other teeth except tooth number 24 are present in the patient's mouth. The patient desires a new tooth in the empty "number 24" space in her mouth.

A full thickness mucoperiosteal flap surgery is utilized to expose the bone in the number 24 space. A slight tissue reflection into the number 23 tooth and number 25 tooth areas is carried out to insure adequate working conditions.

A Midwest Quietair handpiece (or other off-the-shelf handpiece) utilizing a #701XXL bur (Dentsply Midwest of Des Plaines, Illinois) (a #700, #557, #558, etc. bur can be utilized if desired) is used to excavate an implant opening or site in the bone. The implant opening is placed midway between the roots of the number 23 and number 25 teeth. The opening ends at a depth which is about fifteen millimeters and which approximates the depth of the apices of the roots of the number 23 and number 25 teeth. Care is taken not to perforate either the buccal or lingual wall of the bone. In addition, care is taken not to perforate or invade the periodontal ligament space of teeth numbers 23 and 25.

An interrupted drilling technique is utilized to avoid overheating the bone when the #701XXL bur is utilized to form the implant opening. During a drilling sequence, the drill is operated in five second increments and the handpiece is permitted to stall. Light pressure and a gentle downward stroke are utilized.

**PAGE 62, LINES 1–10**

**EXAMPLE 36**

Example 18 is repeated except that the patient is a 55 year old Caucasian male, and the genetic carrier solution is injected into two sites in the coronary artery of the patient. The first site is on the exterior wall on one side of the artery. The second site is inside the wall of the artery on the other side of the artery. A section of the artery is damaged, is partially blocked, and has a weakened wall. The first and second sites are each below the damaged section of the artery. Similar results are obtained, i.e., a new section of artery grows integral with the original artery, and a new section of artery grows adjacent the original artery. The new section of artery has integrated itself at either end with the original artery so that blood flows through the new section of artery.

# THIRD SUPPLEMENTAL DECLARATION

# EXHIBIT C

# CLAIMS

# EXHIBIT C

## CLAIMS
### APPLICATION SERIAL NO. 09/836,750

| | |
|---|---|
| Claim 236 | A method of growing a new portion of a pre-existing heart comprising the steps of placing a growth factor in a body of a human patient and growing new cardiac muscle and growing a new artery in said heart. |
| Claim 238 | The method of claim 236, further comprising repairing a dead portion of said heart. |
| Claim 239 | The method of claim 236, further comprising repairing a damaged portion of said heart. |
| Claim 243 | The method of claim 236, wherein said growth factor comprises a member selected from the group consisting of cells, cellular products, and derivatives of cellular products. |
| Claim 244 | The method of claim 243, wherein said growth factor comprises a cell. |
| Claim 245 | The method of claim 244, wherein said cell is multifactorial and non-specific. |
| Claim 246 | The method of claim 245, wherein said cell comprises a stem cell. |
| Claim 247 | The method of claim 236, wherein said growth factor is placed in said patient by injection. |
| Claim 248 | The method of claim 247, wherein said injection is intravenous. |
| Claim 249 | The method of claim 247, wherein said injection is intraluminal. |
| Claim 250 | The method of claim 247, wherein said injection is intramuscular. |

Claim 251 The method of claim 236, wherein said growth factor is placed in said patient by a carrier.

Claim 252 The method of claim 251, wherein said carrier comprises an angioplasty balloon.

Claim 253 The method of claim 236, wherein said growth factor comprises a gene and a cell.

Claim 257 The method of claim 236, wherein said growth factor is locally placed in said body.

Claim 258 The method of claim 238, wherein said growth factor is locally placed in said body.

Claim 259 The method of claim 239, wherein said growth factor is locally placed in said body.

Claim 260 The method of claim 243, wherein said growth factor is locally placed in said body.

Claim 261 The method of claim 236, wherein said growth factor comprises living stem cells harvested from bone marrow.

Claim 262 The method of claim 238, wherein said growth factor comprises living stem cells harvested from bone marrow.

Claim 263 The method of claim 239, wherein said growth factor comprises living stem cells harvested from bone marrow.

Claim 264 A method of growing a new portion of a pre-existing heart comprising locally placing a growth factor comprising a stem cell in a body of a human patient to grow new cardiac muscle in said heart.

Claim 265 The method of claim 264, wherein said stem cell is placed in said patient by injection.

Claim 266 The method of claim 264, wherein said stem cell comprises living stem cells harvested from bone marrow.

Claim 267 The method of claim 266, wherein said stem cell is placed in said patient by injection.

Claim 268 The method of claim 262, wherein said stem cell is placed in said patient by injection.

Claim 269 The method of claim 263, wherein said stem cell is placed in said patient by injection.

Claim 270 The method of claim 258, wherein said growth factor comprises a cell and said cell is placed adjacent to said dead portion of said heart.

Claim 271 The method of claim 259, wherein said growth factor comprises a cell and said cell is placed adjacent to said damaged portion of said heart.

Claim 272 The method of claim 265, wherein said stem cell is injected into said heart.

Claim 273 The method of claim 267, wherein said stem cell is injected into said heart.

Claim 274 The method of claim 238, wherein said growth factor comprises a cell and said cell is placed in said body by intravenous injection.

Claim 275 The method of claim 239, wherein said growth factor comprises a cell and said cell is placed in said body by intravenous injection.

Claim 276 The method of claim 238, wherein said growth factor comprises a cell and said cell is placed in said body by intraluminal injection.

Claim 277 The method of claim 239, wherein said growth factor comprises a cell and said cell is placed in said body by intraluminal injection.

Claim 278 The method of claim 238, wherein said growth factor comprises a cell and said cell is placed in said body by an angioplasty balloon.

Claim 279 The method of claim 239, wherein said growth factor comprises a cell and said cell is placed in said body by an angioplasty balloon.

Claim 280 The method of claim 236 further comprising determining blood flow through said newly grown artery.

Claim 281 The method of claim 238 further comprising determining blood flow through said newly grown artery.

Claim 282 The method of claim 239 further comprising determining blood flow through said newly grown artery.

Claim 283 The method of claim 236 further comprising observing said newly grown artery.

Claim 284 The method of claim 238 further comprising observing said newly grown artery.

Claim 285 The method of claim 239 further comprising observing said newly grown artery.

Claim 286 A method of repairing a dead portion of a pre-existing heart comprising the steps of placing stem cells adjacent said dead portion; forming a new artery in said heart, thereby causing said dead portion of said heart to be repaired.

Claim 287 The method of claim 286, wherein said stem cells are placed by injection.

Claim 288 The method of claim 286, wherein said stem cells are placed by intraluminal administration.

Claim 289 The method of claim 286, wherein said stem cells are placed by an angioplasty balloon.

Claim 290 A method of repairing a damaged portion of a pre-existing heart comprising the steps of placing stem cells adjacent said damaged portion; forming a new artery in said heart, thereby causing said damaged portion of said heart to be repaired.

Claim 291 The method of claim 290, wherein said stem cells are placed by injection.

Claim 292 The method of claim 290, wherein said stem cells are placed by intraluminal administration.

Claim 293 The method of claim 290, wherein said stem cells are placed by an angioplasty balloon.

# EXHIBIT E

**Caplan 1991 publication**
**Journal of Orthopaedic Research**
**entitled "Mesenchymal Stem Cells"**

*Journal of Orthopaedic Research*
9:641–650 Raven Press, Ltd., New York


# Mesenchymal Stem Cells*

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Summary: Bone and cartilage formation in the embryo and repair and turnover in the adult involve the progeny of a small number of cells called mesenchymal stem cells. These cells divide, and their progeny become committed to a specific and distinctive phenotypic pathway, a lineage with discrete steps and, finally, end-stage cells involved with fabrication of a unique tissue type, e.g., cartilage or bone. Local cuing (extrinsic factors) and the genomic potential (intrinsic factors) interact at each lineage step to control the rate and characteristic phenotype of the cells in the emerging tissue. The study of these mesenchymal stem cells, whether isolated from embryos or adults, provides the basis for the emergence of a new therapeutic technology of self-cell repair. The isolation, mitotic expansion, and site-directed delivery of autologous stem cells can govern the rapid and specific repair of skeletal tissues. Key Words: Mesenchymal stem cells—Bone—Cartilage—Differentiation—Self-cell therapy—Skeletal tissue—Embryo—Adult.

## THE CONCEPT

It is generally agreed that in an embryo a mesenchymal stem cell is a pluripotent progenitor cell which divides many times and whose progeny eventually gives rise to skeletal tissues: cartilage, bone, tendon, ligament, marrow stroma, connective tissue (Fig. 1). By definition, these stem cells are not governed by or limited to a fixed number of mitotic divisions. Their progeny are affected by a number of factors, however, as they become tracked into very specific developmental pathways in which both intrinsic and extrinsic factors combine to control the molecular and cellular pattern of expression that results in specific tissues that perform specific functions based on their molecular repertoire (9,11).

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Indeed, the progression from stem cell to final end phenotype is marked by discrete stages with transit from one stage to the next dependent on local cuing from surrounding cells (paracrine regulation) as well as signals emitted by the cell itself and the reception of its own signaling (autocrine regulation) (10,57). The sum of these various intrinsic and extrinsic signals defines the developmental position of the cells. Although difficult to reconstruct on a cell culture dish, such "positional information" has been experimentally approached by studying embryonic cells in culture, cells that have the potential to differentiate into various phenotypes (7,9,11,15).

The concept of stem cells is now well established (21,60). Two systems serve as models for such a concept: First, *Caenorhabditus elegans* is a small worm whose entire developmental lineage map has been described (21); every cell found in the adult has been carefully tracked and its progenitor tree precisely established with every branch and subbranch delineated. Second, and to be emphasized, the heamtopoietic cell lineage has been described with its several diverging pathways (21,52). It is now clear that each separate pathway and, indeed,

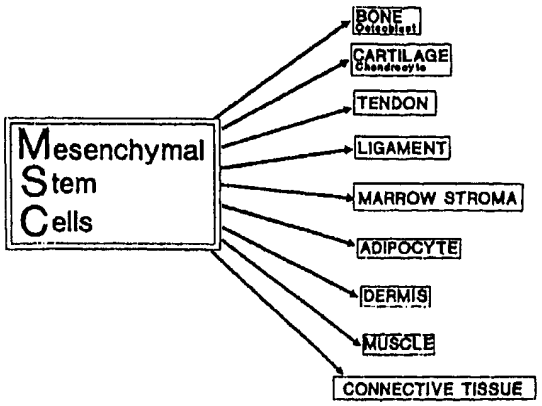


FIG. 1. Mesenchymal stem cell phenotypes. Mesenchymal stem cells are theoretically capable of differentiating through a series of separate and unique lineage transitions into a variety of end-stage phenotypes as shown.

progression through each separate stage within a discrete pathway is controlled by a balance of extrinsic and intrinsic macromolecules. Molecular biologists continue to isolate, clone, and express large amounts of these proteins, which allows use of cell culture systems to identify accurately the factor that controls progression to which stage and when (51,70). The challenge for skeletal biologists is to use the new information and new molecular tools to translate these advances into a better understanding of skeletal development, physiology, and repair.

## EMBRYONIC MESENCHYMAL CELLS

The middle embryonic layer, the mesoderm, gives rise to all of the body's skeletal elements.* The term, mesenchyme, is derived from the Greek meaning "middle" (meso) "infusion" and refers to the ability of mesenchymatous cells to spread and migrate in early embryonic development between the ectodermal and endodermal layers. This characteristic migratory, space-filling ability is the key element of all wound repair in adult organisms involving mesenchymal cells in skin (dermis), bone (periosteum), or muscle (perimysium). Proteins that serve as chemoattractants, chemicals that specifically encourage this migratory activity to wound or developmental sites have been identified (24,32,59). The migratory activity of mesenchymal cells is complemented by their capacity to aggregate specifically to form unique developmental structures or, in adults, to form repair blastemas, which are then capable of responding to local cues and differentiating accordingly to achieve regenerative repair (10,11).

* For the sake of clarity, I address only issues related to cartilage or bone, although the same general experimental approach and logic can be used for other mesenchymal tissues.

### Chick Limb Cells

More than 20 years ago, my collaborators and I attempted to define experimentally the conditions and cues necessary to control the differentiation of embryonic mesenchymal cells into cartilage and bone (5,7,17). Both in vivo and in vitro studies were used, but development of cell cultures and the general approach of using cell cultures has provided the experiential basis for approaching the study of mesenchymal stem cells from adults. The system we developed was the culturing of stage 24 (day 4.5) embryonic chick limb mesenchymal cells under conditions that promoted differentiation of cartilage (chondrocytes) (5,7,13,20) and bone (osteoblasts) (42,65).

#### *Chondrocytes*

Our first experimental effort with embryonic chick limb mesenchymal cells was to focus on chondrocyte development, which we learned was controlled by the initial plating density (5,17), oxygen levels (14), or, as recently shown by other investigators, a variety of physical and chemical factors (53,58,61). The key factor in the conversion of a mesenchymal cell to a chondrocyte is maintaining the progenitor cell in a round, unspread conformation. This can be accomplished simply by plating the cells initially under very compact, high-density conditions: $5 \times 10^6$ embryonic stage-24 limb mesenchymal cells per 35-mm dish (5,17). Even in a simple, defined medium consisting of insulin, transferrin, bovine serum albumin (BSA), and hydrocortisone in Eagle's minimum essential medium (MEM), the differentiation of chondrocytes and their further development can be documented as long as the cells are initially seeded at high density (18,30).

The high-density, limb cell-derived chondrocyte in culture makes two cartilage-specific molecules in abundance: type II collagen (68) and a large chondroitin sulfate, keratan sulfate proteoglycan (CSPG) (13,18,20). By detailed chemical and physical characterization of the CSPG synthesized on each day of culture, we showed that the glycosaminoglycan chains are biosynthesized slightly differently with

time (Fig. 2). Peptide maps show that the newly synthesized core protein (26) is identical on each day of culture, whereas the chondroitin sulfate chains are synthesized progressively shorter (30,000 D on day 2 to 15,000 D on day 20) and the keratan sulfate chains are synthesized progressively larger (0 to 10,000 D) (13,20). This biosynthetic progression is exactly what has subsequently been shown to occur in the cartilages of embryonic, adult, and aging human (50) and bovine specimens (62).

That embryonic chondrocytes have an aging-dependent program of changing biosynthesis is further documented when cultured embryonic chick chondrocytes are transplanted in a fibrin-based delivery vehicle into defects at the articular surface of adult chickens (29). Such chondrocytes produce what appears to be appropriate cartilaginous matrix and have been followed >18 months. The resulting repair cartilage appears to integrate perfectly into the defect and to provide the animal with a healthy, normal articular surface. These experiments and others clearly establish the concept of repairing cartilage with embryonic or appropriate reparative cells.

### *Osteoblasts*

Our initial success in studying emergence of chondrocytes and formation of cartilaginous tissue from cultures of limb mesenchymal cells encouraged us to study differentiation of osteoblasts and formation of bone as well. Our initial logic was that high-density conditions caused cartilage formation and that cartilage was the progenitor tissue of bone. (Some investigators have reported that cartilage provides the scaffold for bone formation.) After 2 years of frustrating experimentation, we realized that when infrequent bone and osteoblasts could be identified, the bone had formed at a distance from cartilage and never on or in the cartilage (42). By carefully decreasing the initial cell density of limb mesenchymal cells to just below the density at which some mineralized cartilage could form ($2 \times 10^6$ cells/35-mm dish), we observed numerous deposits of bone and abundant osteoblasts which, again, were clearly at some distance from cartilage (6,42,44). In addition, these osteoblasts exhibited the classic response to parathyroid hormone (PTH) of elevated cyclic AMP levels (71,72) and possessed a bone-specific alkaline phosphatase (43). These studies clearly indicated that embryonic chick limb mesenchymal cells were capable of differentiating into osteoblasts and that the culture conditions supporting optimum osteoblast emergence were different from the conditions optimum for chondrogenesis.

### Mouse and Human Limb Cells

With regard to cartilage and bone, the properties of mouse and human limb mesenchymal cells in culture appear to be quite similar, if not identical (25,46). Likewise, cartilage and bone development in vivo are also quite comparable, with the major exception that embryonic cartilage of chick does not calcify whereas that of mammals always calcifies (16). The comparable developmental properties of aves, rodents, and humans encourages us to continue experimentation with animal cells as an approximation of better understanding of the properties of human cells and tissues.

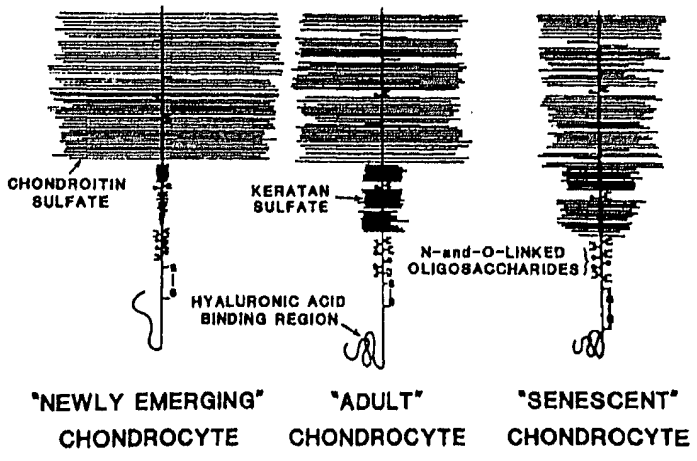


**FIG. 2.** Proteoglycans synthesized by newly differentiated, mature, and senescent chondrocytes. With increasing age, chondrocytes synthesize proteoglycans that have smaller chondroitin sulfate chains and larger keratan sulfate chains (7,8,12,13,20).

## LINEAGE OF MESENCHYMAL CELLS

### Cartilage

The important inference from the above discussion is that chondrocytes have a programmed (i.e., genetically dictated) sequence of changes in their end-stage expression (8,12). The differences in glycosaminoglycan chain lengths or chemistry are stable to cell culturing or metabolic perturbation. The control of these events is not known, but all experiments designed to slow this sequence of biosynthetic alterations or reverse them have failed. The inference is that a genomic mechanism somehow "tells time" and that this clock is hard-wired and unidirectional (8,12).

Such biosynthetic changes in articular cartilage are different from the lineage changes observed in adult growth plate or embryonic limb cartilage. A discrete set of expressional stages or lineage states, comprising dividing, maturing, and hypertrophic chondrocytes, is apparent in embryonic limb tissue, cell culture (13,58,61), and in the growth plate (19,28). Eventually, the hypertrophic cartilage in vivo is eroded by vascular, marrow, and phagocytic cells and replaced by bone. Each chondrocytic lineage state is uniquely different from its predecessor, as shown in Fig. 3. For example, hypertrophic chondrocytes synthesize a unique small collagen, type X, and a unique proteoglycan (54,55); neither of these molecules is synthesized by mature chondrocytes. In this particular circumstance, several factors are proposed to contribute to conversion of mature chondrocytes to hypertrophic chondrocytes (35); reversal of this process has not been reported.

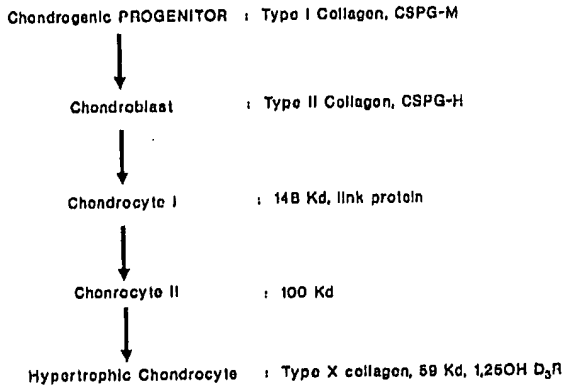


**FIG. 3.** Chondrogenic lineage. Based on the experiments of Solursh et al. (58,61) a hypothetical lineage map can be constructed to consist of at least five separate stages based on the changing biosynthesis of proteins (named or by molecular weight, $K_d$) or chondroitin sulfate proteoglycan (CSPG). The receptor for 1,25-dihydroxy Vitamin $D_3$ is represented as $1,25OHD_3R$.

### Bone

We recently reviewed the major aspects of embryonic bone development. Figure 4 shows several important elements or rules governing this complex process (10,11,16). First, a discrete positioning of progenitor cells, stacked cells, existed in proximity to the developing bone (47). The stacked cells give rise to osteoblats in a discrete series of lineage steps (described below). The end stage or secretory osteoblast is positioned by its proximity to vasculature, with the "back" of the osteoblast to the capillary and osteoid deposited from the "front" of this highly oriented secretory cell (47,48). The vasculature is the orientor of osteogenesis and the osteoblast is the formative element. Cartilage is not replaced by bone, but is instead the target for vascular (marrow) replacement (48); in the early limb, the cartilage model exactly defines the eventual marrow cavity.

That a discrete series of individual lineage stages exists between the progenitor cells in the stacked cell layer and the secretory osteoblasts is now clear, as shown in Fig. 5. We recently isolated four monoclonal antibodies, SB1, 2, 3, and 5, which have helped provide evidence for an osteoblast lineage (3,4). Progenitor cells in the stacked cell layer and osteocytes do not interact with SB1, 2, or 3. Newly differentiated osteogenic cells react with SB1, but not with SB2 or 3, whereas fully secretory osteoblasts react with SB1, 2, and 3. A subpopulation of osteogenic cells reacts with SB2, but not SB3. Osteocytes react with OB7.3 of Nijweide and Mulder (38) or with our SB5, but not with SB1, 2, or 3. The lineage tree in Fig. 3 is based on these observations and not only establishes the existence of an osteoblastic lineage but suggests that osteocytes are derived directly from osteoblasts with SB1, 2, and 3 antigens that are suppressed as SB5 and OB7.3 are turned on. Experiments are now in progress to use these monoclonal antibodies to isolate representatives of each lineage stage so that studies can be conducted to identify the agents that promote the progression from one lineage stage to the next. Central to the thesis presented below is the existence of osteoprogenitor cells in the stacked cell layer, the future periosteum.

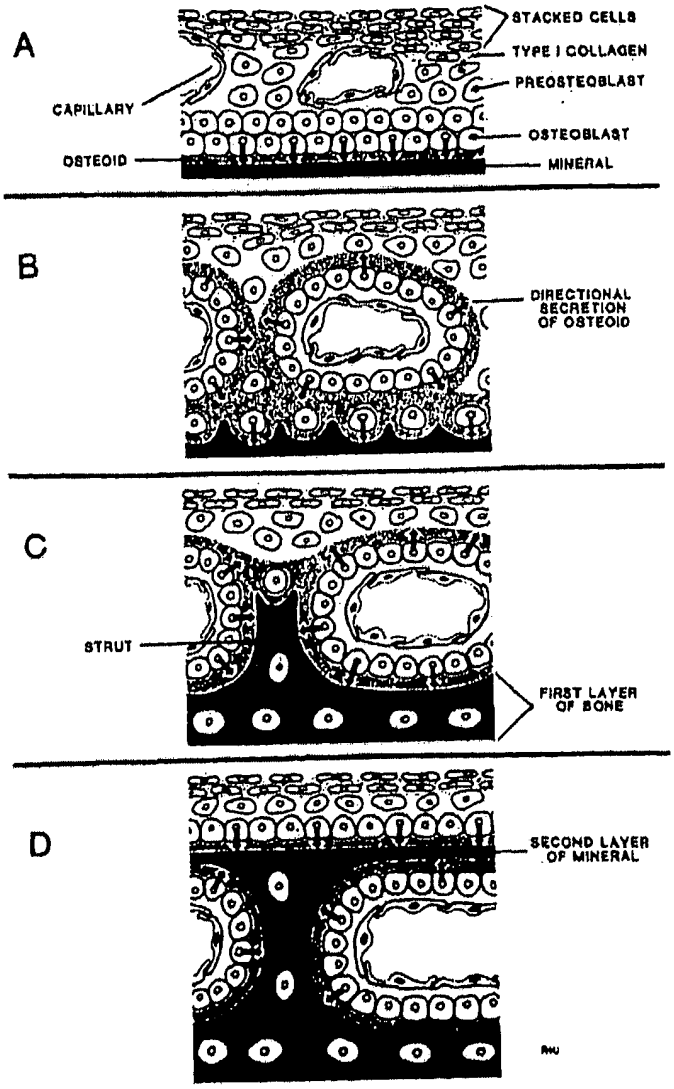


FIG. 4. Sequence of progressive in vivo bone development. Progressive repositioning of the vasculature from outside the stacked cell layer to a position in close approximation to the first layer of secretory osteoblasts responsible for formation of the first bony collar of the chick tibia (11,47,48). The osteoblast is oriented with its back toward the invading capillary and secretion of osteoid toward the cartilage core from the osteoblast's face. In this model, osteoblasts secrete osteoid in a direction away from vasculature (B), causing formation of a strut (C) and eventually forming the second layer of bone (D). These observations show that an intimate relationship exists between vasculature and newly forming bone.

## BIOACTIVE FACTORS IN BONE

From the earliest days of modern humans, bone has been recognized to have the powerful capacity to repair discontinuities (22). A variety of bioactive factors combine in a complex multicellular, multistep response in which reparative cells are specifically attracted to the repair site. These cells then aggregate, multiply, bridge the bone gap, and differentiate into chondrocytes or osteoblasts as controlled by the proximity to vasculature. Recently, an intensive research activity to identify and characterize these various bioactive factors was largely successful (56,66,67,69). Our laboratory has described the purification of a protein factor, chondrogenic stimulating activity (CSA), which converts embryonic limb mesenchymal cells to chondrocytes (63,64). We are also attempting to purify a bone-derived chemoattractant for mesenchymal cells by using the now standard modified Boyden chamber (31,33).

Relevant to the thesis developed below, the identity and manipulation of the cells responding to bone-derived bioactive factors is directly related to successful bone repair. Such responding cells are present in the adult periosteum (36), dermis (49),

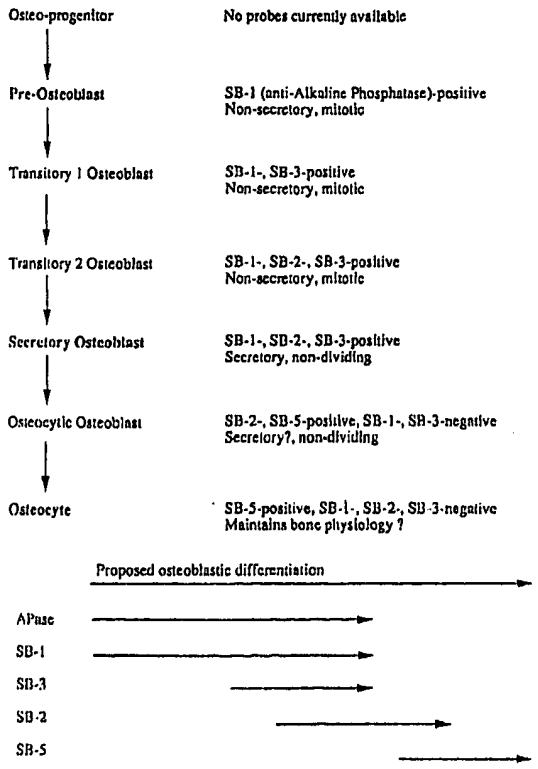


FIG. 5. Osteogenic cell lineage. Based on recent experimentation in which monoclonal antibodies were generated to cell surface antigens of osteogenic cells (3,4), a tentative lineage diagram reflects acquisition or loss of specific antigenic determinants. The characteristics of SB1, 2, and 3 were described previously (3); SB-5 (4) has been characterized and is similar to OB7.3 of Nijweide and Mulder (38). The individual lineage states are not weighted in terms of their prevalence or dwell-time; e.g., "transitory osteoblast 1" occurs rarely and cannot be recognized easily except at specific times and locations, whereas the "secretory osteoblast" is easily recognized and plentiful.

bone marrow (1,40,41,45), and connective tissue associated with muscle (34,37). One or all of these repositories are capable of forming bone when appropriately delivered bioactive factors are presented.

Alternately, when the responsive cells, stem cells, are placed in suitable delivery vehicles that can retain these cells while encouraging vascular invasion, bone can be observed to form. Recently, we used calcium phosphate porous ceramics in composite with marrow to encourage bone formation at both heterotopic and orthotopic sites (40,41). Whole disaggregated marrow cells in suspension are loaded into porous ceramic and transplanted to subcutaneous, intramuscular, or bone defect sites in vivo. In 1–2 months, the few mesenchymal stem cells in the marrow have replicated massively and differentiated into osteoblasts. In the dead-end pores of the ceramic, which are devoid of vasculature, these stem cells differentiate into chondrocytes and form cartilage.

## MESENCHYMAL STEM CELLS

From the above discussion several key facts are evident. First, embryonic mesenchymal stem cells in the limb which give rise to cartilage and bone in vivo can be manipulated in vitro. Second, these cells have a lineage progression of separate, individual steps, whether it be the chondrogenic or osteogenic pathway. Third, local cuing, sometimes involving highly potent protein factors, is responsible for providing positional information and causing lineage progression. Cell culture conditions have been refined to the extent that not only can these progressive events be studied in detail, but manipulation of the cells is also possible to provide control of tissue size and function.

Fourth, although chondrocytes and osteoblasts are derived from a common mesenchymal cell, the conditions for their initial differentiation and progression through the individual steps of their lineages are uniquely different. For example, osteogenesis is dependent on proximity to vasculature whereas chondrogenesis requires the complete absence of vasculature (7,10,11,16); osteogenesis is optimum at an initial cell culture seeding density in 35-mm dishes of $2 \times 10^6$ embryonic limb mesenchymal cells, whereas chondrogenesis is optimum at $5 \times 10^6$ cells (5,17,42).

Fifth, bone forms from mesenchymal stem cells in a cartilage-independent manner with vasculature providing a determinative discriminator between these two tissues; embryonic cartilage is not replaced by bone, but rather by vasculature and marrow (10,11,16). Sixth, we can demonstrate that three tissue sites are the repositories of mesenchymal stem cells: marrow (1,40,41,45), periosteum (36), and muscle connective tissue (34,37).

## MARROW

Figure 6 outlines an assay to demonstrate that marrow contains mesenchymal stem cells capable of differentiation into cartilage and bone. Whole marrow is disrupted into single cells by passing it through needles of successively smaller sizes; the

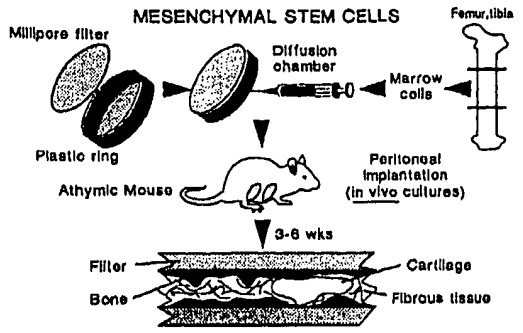


FIG. 6. Diffusion chamber assay in nude mice. Cell samples from marrow or other sources can be loaded into chambers composed of two Millipore filters glued to the edges of a plastic ring. These chambers are then implanted in the peritoneal cavity of athymic (nude) mice as a highly vascular in vivo incubation site. The filters prevent host cells from entering the chambers but permit rapid diffusion of nutrients and other factors into or out of the chamber. Histologic identification of two distinctive phenotypes, cartilage and bone, indicates that mesenchymal stem cells were present in the initial inoculum (1,2,45).

cells are counted, and $1–10 \times 10^6$ cells are placed in a small diffusion chamber (1,2,45). This chamber is of simple construction consisting of a small plastic ring onto which two Millipore filters have been glued. The filters allow body fluids (salts, nutrients, proteins, large protein complexes) to pass in and out of the chamber, but cells inside are not mixed with host cells, and tissues such as the vasculature are completely excluded. These chambers are implanted into the peritoneal cavity of an athymic (nude) mouse as an in vivo incubator, and they quickly become surrounded by host vasculature. Detailed studies have shown that the hematopoietic cells are eliminated, whereas mesenchymal cells vigorously divide and differentiate into cartilage in the middle of the chamber and bone at the filter interfaces closest to the enveloping vasculature (1,2,45). The presence of both cartilage and bone in the diffusion chamber has been compared to the presence of predominantly bone inside the highly vascularized pore regions of porous calcium phosphate ceramics loaded with marrow cells and implanted at heterotopic or orthotopic sites described above (40,41).

As a refinement of these experiments, we have been able to purify marrow mesenchymal cells by their differential adhesion to culture dishes and have successfully cultured cells through many passages (23). These cultured marrow mesenchymal cells from rat or chicken retain their capacity to differentiate into osteoblasts in ceramics through such subculturing. Of importance is the demonstrated success of isolating marrow mesenchymal cells and mitotically expanding these cells with retention of their full developmental potency to differentiate into osteoblasts or chondrocytes.

### Periosteum

Another repository for mesenchymal stem cells is the periosteum, a complex layer of cells that composes the outermost layer of long bone; we have termed the periosteum the stacked cell layer in developing embryos (1,16,47,48). This layer clearly responds to injury by rapidly expanding and forming woven bone; it also has cells capable of differentiating into chondrocytes when the periosteum is transplanted into an articular cartilage defect (39). In experimentation paralleling that described above for marrow mesenchymal cells,we have been successful in culturing and passaging periosteal cells (36). In porous ceramics implanted in nude mice, these cultured periosteal cells differentiate into osteoblasts (36). When the same cell preparation is injected into a subcutaneous site in a nude mouse, the cultured periosteal cells differentiate into both bone and cartilage (36). The important point is that culture-expanded periosteal cells retain their full developmental potency and can be manipulated to form two very complex and different tissues, bone or cartilage.

## THE FUTURE: (SELF-CELL THERAPY)

Several important conceptual and technical advances have converged to allow us to consider the possibility of using a patient's own mesenchymal stem cells as starting material for tissue repair protocols. Mesenchymal stem cells must exist to maintain the living organisms, just as hematopoietic stem cells must exist to support both red and white blood cell turnover. Developmental biology has taught us that differentiated cells arise in a sequence of definitive cellular and molecular transitions, a lineage, from stem cell to end phenotype. Bone, for example, turns over; new osteoblasts arise, have a defined half-life, make new bone, and then die, to be replaced by other newly differentiating end-stage osteoblasts. Such osteoblasts must arise from stem cells; thus, a living organism must have repositories of stem cells.

Therefore, we might be able to isolate such human mesenchymal stem cells and place them in cell

culture, where we could mitotically expand their numbers. Eventually, if we had enough of these cells, we could reintroduce them into the original donor in a manner that guaranteed that they would massively differentiate into a specific tissue, such as cartilage or bone, at a transplantation or repair site. Immunorejection would not be a problem because the donor and host would be one and the same.

The first experimental step to test this idea is to determine if the animal-based technology described above can be modified to be used with human material. The first attempts at this have been highly encouraging. Recently, human marrow was introduced into diffusion chambers which were placed in nude mice; both cartilage and bone were eventually observed in the chamber (2). We recently cultured human marrow and isolated mesenchymal cells that were passaged, introduced into porous ceramics, and implanted subcutaneously in nude mice. In the pore regions of these highly vascularized composites, bone clearly formed in every sample of culture-expanded, marrow-derived mesenchymal cells tested (27). These preliminary experiments provide hope that the animal-based technology developed for mesenchymal cells from marrow or periosteum will be translatable to humans.

The concept of ex vivo manipulation of cells and their reimplantation into a donor is the basis for proposing self-cell therapy as a future possibility. Massive bone regeneration to fill gaps from tumor excision, regeneration of damaged articular cartilage, and maintenance of bone formation in the elderly at risk for osteoporosis are clinical protocols that require large numbers of the appropriate reparative skeletal cells. The patient's own mesenchymal stem cells may prove to be the basis of a new, cell-based treatment plan requiring the merging of molecular biology to produce specific bioactive factors, cell biology to develop ex vivo manipulation regimens, and surgeons able to implant cells capable of repairing skeletal defects by the regeneration process.

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# EXHIBIT F

**Caplan 2001 publication**

1: Trends Mol Med. 2001 Jun;7(6):259-64.

ELSEVIER FULL-TEXT ARTICLE



# Mesenchymal stem cells: building blocks for molecular medicine in the 21st century.

Caplan AI, Bruder SP.

Dept of Biology, Skeletal Research Center, Case Western Reserve University, Cleveland, OH, USA.

Mesenchymal stem sells (MSCs) are present in a variety of tissues during human development, and in adults they are prevalent in bone marrow. From that readily available source, MSCs can be isolated, expanded in culture, and stimulated to differentiate into bone, cartilage, muscle, marrow stroma, tendon, fat and a variety of other connective tissues. Because large numbers of MSCs can be generated in culture, tissue-engineered constructs principally composed of these cells could be re-introduced into the in vivo setting. This approach is now being explored to regenerate tissues that the body cannot naturally repair or regenerate when challenged. Moreover, MSCs can be transduced with retroviral and other vectors and are, thus, potential candidates to deliver somatic gene therapies for local or systemic pathologies. Untapped applications include both diagnostic and prognostic uses of MSCs and their descendents in healthcare management. Finally, by understanding the complex, multistep and multifactorial differentiation pathway from MSC to functional tissues, it might be possible to manipulate MSCs directly in vivo to cue the formation of elaborate, composite tissues in situ.

Publication Types:
- Review
- Review, Tutorial

PMID: 11378515 [PubMed - indexed for MEDLINE]

# EXHIBIT I

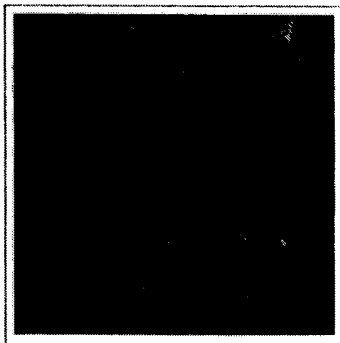
**"Cell (biology)" from Wikipedia, the free encyclopedia**

# Cell (biology)

From Wikipedia, the free encyclopedia
(Redirected from Biological cell)
You have new messages (last change).

It has been suggested that *extracellular* be merged into this article or section. (Discuss)

The **cell** is the structural and functional unit of all living organisms, and is sometimes called the "building block of life." Some organisms, such as bacteria, are unicellular, consisting of a single cell. Other organisms, such as humans, are multicellular, (humans have an estimated 100 trillion or $10^{14}$ cells; a typical cell size is 10 μm, a typical cell mass 1 nanogram).



Cells in culture, stained for keratin (red) and DNA (green).

The cell theory, first developed in 1839 by Schleiden and Schwann, states that all organisms are composed of one or more cells; all cells come from preexisting cells; all vital functions of an organism occur within cells, and cells contain the hereditary information necessary for regulating cell functions and for transmitting information to the next generation of cells.

The word *cell* comes from the Latin *cella*, a small room. The name was chosen by Robert Hooke when he compared the cork cells he saw to the small rooms monks lived in.

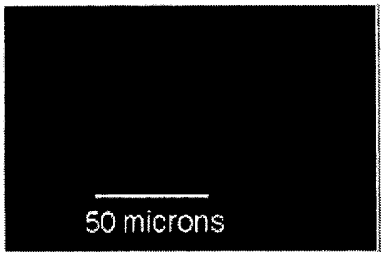
Some (Lynn Margulis and Dorian Sagan, 1995) have argued that the cell is the smallest unit of life.

## Contents

# Overview

## Properties of cells

Each cell is at least somewhat self-contained and self-maintaining: it can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary. Each cell stores its own set of instructions for carrying out each of these activities.



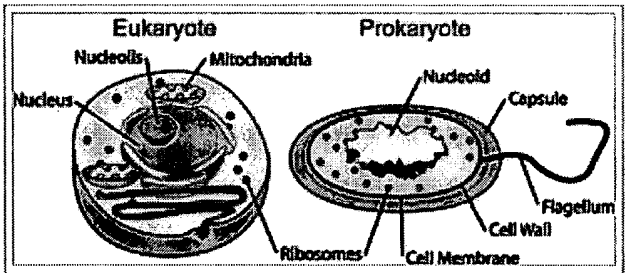

Mouse cells grown in a culture dish. These cells grow in large clumps, but each individual cell is about 10 micrometres across.

All cells share several abilities:

Reproduction by cell division (mitosis or meiosis).
Metabolism, including taking in raw materials, building cell components, converting energy, molecules and releasing by-products. The functioning of a cell depends upon its ability to extract and use chemical energy stored in organic molecules. This energy is derived from metabolic pathways.
Synthesis of proteins, the functional workhorses of cells, such as enzymes. A typical mammalian cell contains up to 10,000 different proteins.
Response to external and internal stimuli such as changes in temperature, pH or nutrient levels.
Traffic of vesicles.

# Subcellular components

All cells, whether prokaryotic or eukaryotic, have a membrane, which envelopes the cell, separates its interior from its environment, controls what moves in and out, and maintains the electric potential of the cell. Inside the membrane, a salty cytoplasm takes up most of the cell volume. All cells possess DNA, the hereditary material of genes, and RNA, containing the information necessary to build various proteins such as enzymes, the cell's primary machinery. There are also other kinds of biomolecules in cells. This article will list these primary components of the cell, then briefly describe their function.




The cells of eukaryotes and prokaryotes. This diagram illustrates a typical human cell.

## Cell membrane: A cell's protective coat

*Main article:* Cell membrane

The cytoplasm of a eukaryotic cell is surrounded by a *plasma membrane*. A form of plasma membrane is also found in prokaryotes, but is usually referred to as the *cell membrane*. This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of lipids (hydrophobic fat-like molecules) and hydrophilic phosphorous molecules. Hence the layer is called a phospholipid bilayer. Embedded within this membrane is a variety of other molecules that act as channels and pumps, moving different molecules into and out of the cell.

## Cytoskeleton: A cell's scaffold

*Main article:* Cytoskeleton

The cytoskeleton is an important, complex, and dynamic cell component made up of microfilaments. It acts to organize and maintain the cell's shape; anchors organelles in place; helps during endocytosis, the uptake of external materials by a cell; and moves parts of the cell in processes of growth and mobility. There is a great number of proteins associated with the cytoskeleton, each controlling a cell's structure by directing, bundling, and aligning filaments.

## Genetic material

Two different kinds of genetic material exist: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Most organisms use DNA for their long-term information storage, but some viruses (retroviruses) have RNA as their genetic material. The biological information contained in an organism is encoded in its DNA or RNA sequence. RNA is also used for information transport (e.g., mRNA) and enzymatic functions (e.g., ribosomal RNA) in organisms that use DNA for the genetic code itself.

Prokaryotic genetic material is organized in a simple circular DNA molecule (the bacterial chromosome) in the nucleoid region of the cytoplasm. Eukaryotic genetic material is divided into different, linear molecules called chromosomes inside a discrete nucleus, usually with additional genetic material in some organelles like mitochondria and chloroplasts (see endosymbiotic theory).

A human cell has genetic material in the nucleus (the nuclear genome) and in the mitochondria (the mitochondrial genome). In humans the nuclear genome is divided into 46 linear DNA molecules called chromosomes. The mitochondrial genome is a circular DNA molecule separate from the nuclear DNA. Although the mitochondrial genome is very small, it codes for some important proteins.

Foreign genetic material (most commonly DNA) can also be artificially introduced into the cell by a process called transfection. This can be transient, if the DNA is not inserted into the cell's genome, or stable, if it is.

## Organelles

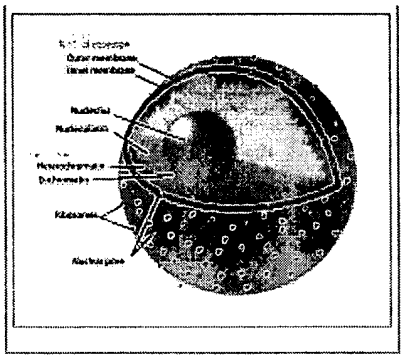
*Main article:* Organelle

The human body contains many different organs, such as the heart, lung, and kidney, with each organ performing a different function. Cells also have a set of "little organs," called organelles, that are adapted and/or specialized for carrying out one or more vital functions. Membrane-bound organelles are found only in eukaryotes.

Cell nucleus (a cell's information center)

The cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis occur. The nucleus is spheroid in shape and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects a cell's DNA from various molecules that could accidentally damage its structure or interfere with its processing. During processing, DNA is transcribed, or copied into a special RNA, called mRNA. This mRNA is then transported out of the nucleus, where it is translated into a specific protein molecule. In prokaryotes, DNA processing takes place in the cytoplasm.

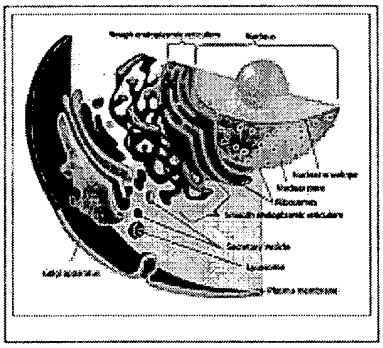
Ribosomes (the protein production machine)



Ribosomes are found in both prokaryotes and eukaryotes. The ribosome is a large complex composed of many molecules, including RNAs and proteins, and is responsible for processing the genetic instructions carried by an mRNA. The process of converting an mRNA's genetic code into the exact sequence of amino acids that make up a protein is called translation. Protein synthesis is extremely important to all cells, and therefore a large number of ribosomes — sometimes hundreds or even thousands — can be found throughout a cell.

Mitochondria and Chloroplasts (the power generators)
Mitochondria are self-replicating organelles that occur in various numbers, shapes, and sizes in the cytoplasm of all eukaryotic cells. As mitochondria contain their own genome that is separate and distinct from the nuclear genome of a cell, they play a critical role in generating energy in the eukaryotic cell, a process involving a number of complex metabolic pathways. Chloroplasts are larger than mitochondria, and convert solar energy into a chemical energy ("food") via photosynthesis. Like mitochondria, chloroplasts have their own genome. Chloroplasts are found only in photosynthetic eukaryotes, like plants and algae. There is a number of plant organelles that are modified chloroplasts; they are broadly called plastids, and are often involved in storage.

Endoplasmic reticulum and Golgi apparatus (macromolecule managers)



The endoplasmic reticulum (ER) is the transport network for molecules targeted for certain modifications and specific destinations, as compared to molecules that will float freely in the cytoplasm. The ER has two forms: the rough ER, which has ribosomes on its surface, and the smooth ER, which lacks them. Translation of the mRNA for those proteins that will either stay in the ER or be *exported* from the cell occurs at the ribosomes attached to the rough ER. The smooth ER is important in lipid synthesis, detoxification and as a calcium reservoir. The Golgi apparatus, sometimes called a *Golgi body* or *Golgi complex* is the central delivery system for the cell and is a site for protein processing, packaging, and transport. Both organelles consist largely of heavily-folded membranes.

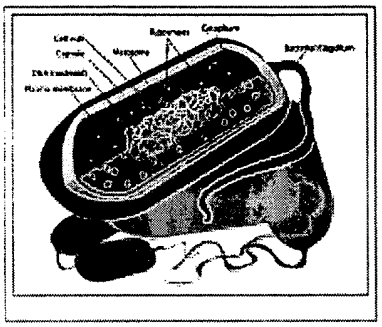
Lysosomes and Peroxisomes (the cellular digestive system)
Lysosomes and peroxisomes are often referred to as the garbage disposal system of a cell. Both organelles are somewhat spherical, bound by a single membrane, and rich in digestive enzymes, naturally-occurring proteins that speed up biochemical processes. For example, lysosomes can contain more than three dozen enzymes for degrading proteins, nucleic acids, and certain sugars called polysaccharides. Here we can see the importance behind compartmentalization of the eukaryotic cell. The cell could not house such destructive enzymes if they were not contained in a membrane-bound system.

Centrioles
Centrioles help in the formation of mitotic appratus. Two centrioles are present in the animal cells. They are also found in some fungi and algae cells.

Vacuoles
Vacuoles store food and waste. Some vacuoles store extra water. They are often described as liquid filled space and are surrounded by a membrane. Some cells, most notably *Amoeba* have contractile vacuoles, which are able to pump water out of the cell if there is too much water.

# Anatomy of cells

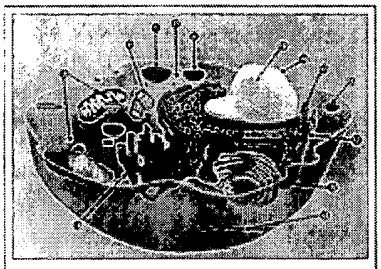
## Prokaryotic cells



Prokaryotes are distinguished from eukaryotes on the basis of nuclear organization, specifically their lack of a nuclear membrane. Prokaryotes also lack most of the intracellular organelles and structures that are characteristic of eukaryotic cells (an important exception is the ribosomes, which are present in both prokaryotic and eukaryotic cells). Most of the functions of organelles, such as mitochondria, chloroplasts, and the Golgi apparatus, are taken over by the prokaryotic plasma membrane. Prokaryotic cells have three architectural regions: appendages called flagella and pili — proteins attached to the cell surface; a cell envelope consisting of a capsule, a cell wall, and a plasma membrane; and a cytoplasmic region that contains the cell genome (DNA) and ribosomes and various sorts of inclusions. Other differences include:

- The *plasma membrane* (a phospholipid bilayer) separates the interior of the cell from its environment and serves as a filter and communications beacon.
- Most prokaryotes have a *cell wall* (some exceptions are *Mycoplasma* (a bacterium) and *Thermoplasma* (an archaeon)). It consists of *peptidoglycan* in bacteria, and acts as an additional barrier against exterior forces. It also prevents the cell from "exploding" (cytolysis) from osmotic pressure against a hypotonic environment. A cell wall is also present in some eukaryotes like fungi, but has a different chemical composition.
- A prokaryotic chromosome is usually a circular molecule (an exception is that of the bacterium *Borrelia burgdorferi*, which causes Lyme disease). Even without a real *nucleus*, the DNA is condensed in a *nucleoid*. Prokaryotes can carry extrachromosomal DNA elements called *plasmids*, which are usually circular. Plasmids can carry additional functions, such as antibiotic resistance.

## Eukaryotic cells



Schematic of a typical animal cell, showing subcellular components. Organelles: (1) nucleolus, (2) nucleus, (3) ribosome, (4) vesicle, (5) rough endoplasmic reticulum (ER), (6) Golgi apparatus, (7) Cytoskeleton, (8) smooth ER, (9) mitochondria, (10) vacuole, (11) cytoplasm, (12) lysosome, (13) centrioles.

There are two types of cells, eukaryotic and prokaryotic. Eukaryotic cells are usually found in multi-cellular organisms, while prokaryotic cells are usually on their own. Eukaryotic cells are about 10 times the size of a typical prokaryote and can be as much as 1000 times greater in volume. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. Most important among these is the presence of a cell nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA. It is this nucleus that gives the eukaryote its name, which means "true nucleus." Other differences include:

The plasma membrane resembles that of prokaryotes in function, with minor differences in the setup. Cell walls may or may not be present.
The eukaryotic DNA is organized in one or more linear molecules, called chromosomes, which are highly condensed (i.e. folded around histones). All chromosomal DNA is stored in the *cell nucleus*, separated from the cytoplasm by a membrane. Some eukaryotic organelles can contain some DNA.

- Eukaryotes can move using *cilia* or *flagella*. The flagella are more complex than those of prokaryotes.

**Table 1: Comparison of features of prokaroytic and eukaryotic cells**

| | Prokaryotes | Eukaryotes |
|---|---|---|
| **Typical organisms** | bacteria, archaea | protists, fungi, plants, animals |
| **Typical size** | ~ 1-10 µm | ~ 10-100 µm (sperm cells, apart from the tail, are smaller) |
| **Type of nucleus** | nucleoid region; no real nucleus | real nucleus with double membrane |
| **DNA** | circular (usually) | linear molecules (chromosomes) with histone proteins |
| **RNA-/protein-synthesis** | coupled in cytoplasm | RNA-synthesis inside the nucleus<br>protein synthesis in cytoplasm |
| **Ribosomes** | 50S+30S | 60S+40S |
| **Cytoplasmatic structure** | very few structures | highly structured by endomembranes and a cytoskeleton |
| **Cell movement** | flagella made of flagellin | flagella and cilia made of tubulin |
| **Mitochondria** | none | one to several dozen (though some lack mitochondria) |
| **Chloroplasts** | none | in algae and plants |
| **Organization** | usually single cells | single cells, colonies, higher multicellular organisms with specialized cells |
| **Cell division** | Binary fission (simple division) | Mitosis<br>Meiosis |

**Table 2: Comparison of structures between animal and plant cells**

| | Typical animal cell | Typical plant cell |
|---|---|---|
| **Organelles** | ▪ Nucleus<br>▪ Nucleolus (within nucleus)<br>▪ Rough endoplasmic reticulum (ER)<br>▪ Smooth ER<br>▪ Ribosomes<br>▪ Cytoskeleton<br>▪ Golgi apparatus<br>▪ Cytoplasm<br>▪ Mitochondria<br>▪ Vesicles<br>▪ Vacuoles<br>▪ Lysosomes<br>▪ Centrioles | ▪ Nucleus<br>▪ Nucleolus (within nucleus)<br>▪ Rough ER<br>▪ Smooth ER<br>▪ Ribosomes<br>▪ Cytoskeleton<br>▪ Golgi apparatus (dictiosomes)<br>▪ Cytoplasm<br>▪ Mitochondrion<br>▪ Vesicle<br>▪ Chloroplast and other plastids<br>▪ Central vacuole<br>▪ Tonoplast (central vacuole membrane)<br>▪ Peroxisome<br>▪ Glyoxysome |
| **Additional structures** | ▪ Cilium<br>▪ Flagellum<br>▪ Plasma membrane | ▪ Plasma membrane<br>▪ Cell wall<br>▪ Plasmodesmata<br>▪ Flagellum (only in gametes) |

# Cell functions

## Cell growth and metabolism

*Main articles:* Cell growth, Cell metabolism

Between successive cell divisions, cells grow through the functioning of cellular metabolism. Cell metabolism is the process by which individual **cells** process nutrient molecules. Metabolism has two distinct divisions:

catabolism, in which the cell breaks down complex molecules to produce energy and reducing power, and anabolism, wherein the cell uses energy and reducing power to construct complex molecules and perform other biological functions. Complex sugars consumed by the organism can be broken down into a less chemically-complex sugar molecule called glucose. Once inside the cell, glucose is broken down to make adenosine triphosphate (ATP), a form of energy, via two different pathways.

The first pathway, glycolysis, requires no oxygen and is referred to as anaerobic metabolism. Each reaction is designed to produce some hydrogen ions that can then be used to make energy packets (ATP). In prokaryotes, glycolysis is the only method used for converting energy. The second pathway, called the Krebs cycle, or citric acid cycle, occurs inside the mitochondria and is capable of generating enough ATP to run all the cell functions.

## Creation of new cells

*Main article:* Cell division

Cell division involves a single cell (called a *mother cell*) dividing into two daughter cells. This leads to growth in multicellular organisms (the growth of tissue) and to procreation (vegetative reproduction) in unicellular organisms.

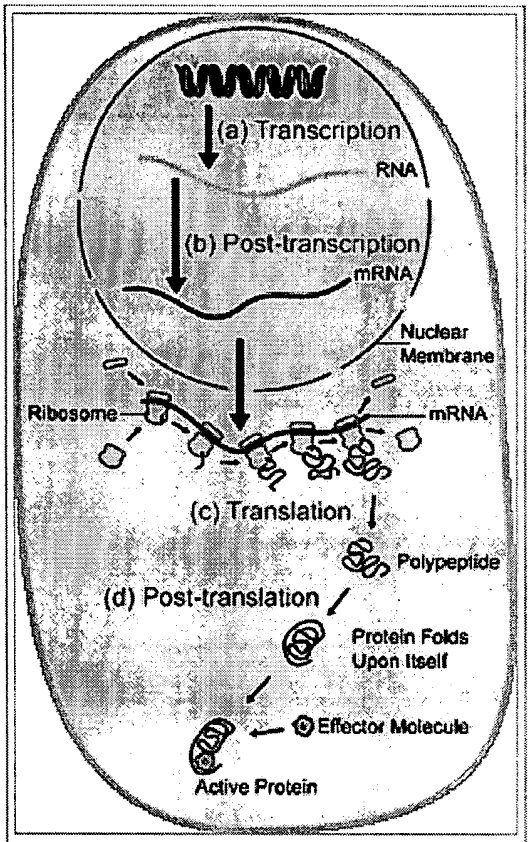
Prokaryotic cells divide by binary fission. Eukaryotic cells usually undergo a process of nuclear division, called mitosis, followed by division of the cell, called cytokinesis. A diploid cell may also undergo meiosis to produce haploid cells, usually four. Haploid cells serve as gametes in multicellular organisms, fusing to form new diploid cells.

DNA replication, or the process of duplicating a cell's genome, is required every time a cell divides. Replication, like all cellular activities, requires specialized proteins for carrying out the job.

## Protein synthesis

*Main article:* Protein biosynthesis

Protein synthesis is the process in which the cell builds proteins. DNA transcription refers to the synthesis of a messenger RNA (mRNA) molecule from a DNA template. This process is very similar to DNA replication. Once the mRNA has been generated, a new protein molecule is synthesized via the process of translation.




An overview of protein synthesis. Within the nucleus of the cell (*light blue*), genes (DNA, *dark blue*) are transcribed into RNA. This RNA is then subject to post-transcriptional modification and control, resulting in a mature mRNA (*red*) that is then transported out of the nucleus and into the cytoplasm (*peach*), where it undergoes translation into a protein. mRNA is translated by ribosomes (*purple*) that match the three-base codons of the mRNA to the three-base anti-codons of the appropriate tRNA. Newly-synthesized proteins (*black*) are often further modified, such as by binding to an effector molecule (*orange*), to become fully active.

The cellular machinery responsible for synthesizing proteins is the ribosome. The ribosome consists of structural RNA and about 80 different proteins. When the ribosome encounters an mRNA, the process of translating an mRNA to a protein begins. The ribosome accepts a new transfer RNA, or tRNA—the adaptor molecule that acts as a translator between mRNA and protein—bearing an amino acid, the building block of the protein. Another site binds the tRNA that becomes attached to the growing chain of amino acids, forming the a polypeptide chain

that will eventually be processed to become a protein.

# Diseases of the cell

## Cancer

Cancer, a class of disease, causes cells to multiply uncontrollably, invading other tissues either by direct growth into adjacent tissue or by implantation into distant sites by metastasis. This uncontrollable multiplication is due to improper replication of the cell's DNA resulting in faulty set of instructions for cell function. Many mutation events may be required to transform a normal cell into a malignant cell. See the main article for details.

# Origins of cells

*Main article:* Origin of life

The origin of cells has to do with the origin of life, and was one of the most important steps in evolution of life as we know it. The birth of the cell marked the passage from prebiotic chemistry to biological life.

## Origin of the first cell

If life is viewed from the point of view of replicators, that is DNA molecules in the organism, cells satisfy two fundamental conditions: protection from the outside environment and confinement of biochemical activity. The former condition is needed to maintain the fragile DNA chains stable in a varying and sometimes aggressive environment, and may have been the main reason for which cells evolved. The latter is fundamental for the evolution of biological complexity. If freely-floating DNA molecules that code for enzymes are not enclosed into cells, the enzymes that benefit a given DNA molecule (for example, by producing nucleotides) will automatically benefit the neighbouring DNA molecules. This might be viewed as "parasitism by default." Therefore the selection pressure on DNA molecules will be much lower, since there is not a definitive advantage for the "lucky" DNA molecule that produces the better enzyme over the others: All molecules in a given neighbourhood are almost equally advantaged.

If all the DNA molecule is enclosed in a cell, then the enzymes coded from the molecule will be kept close to the DNA molecule itself. The DNA molecule will directly enjoy the benefits of the enzymes it codes, and not of others. This means other DNA molecules won't benefit from a positive mutation in a neighbouring molecule: this in turn means that positive mutations give immediate and selective advantage to the replicator bearing it, and not on others. This is thought to have been the one of the main driving force of evolution of life as we know it. (Note. This is more a metaphor given for simplicity than complete accuracy since the earliest molecules of life, probably up to the stage of cellular life, were most likely RNA molecules that acted as both replicators and enzymes: see RNA world hypothesis. However, the core of the reasoning is the same.)

Biochemically, cell-like spheroids formed by proteinoids are observed by heating amino acids with phosphoric acid as a catalyst. They bear much of the basic features provided by cell membranes. Proteinoid-based protocells enclosing RNA molecules could (but not necessarily should) have been the first cellular life forms on Earth.

Another theory holds that the turbulent shores of the ancient coastal waters may have served as a mammoth laboratory, aiding in the countless experiments necessary to bring about the first cell. Waves breaking on the shore create a delicate foam composed of bubbles. Winds sweeping across the ocean have a tendency to drive things to shore, much like driftwood collecting on the beach. It is possible that organic molecules were concentrated on the shorelines in much the same way. Shallow coastal waters also tend to be warmer, further

concentrating the molecules through evaporation. While bubbles comprised of mostly water tend to burst quickly, oily bubbles happen to be much more stable, lending more time to the particular bubble to perform these crucial experiments. The Phospholipid is a good example of a common oily compound prevalent in the prebiotic seas. Phospholipids can be constructed in ones mind as a hydrophilic head on one end, and a hydrophobic tail on the other. Phospholipids also possess an important characteristic, that is being able to link together to form a bilayer membrane. A lipid monolayer bubble can only contain oil, and is therefore not conducive to harbouring water-soluble organic molecules. On the other hand, a lipid bilayer bubble [1] (http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/P/Phospholipids.html) can contain water, and was a likely precursor to the modern cell membrane. If a protein came along that increased the integrity of its parent bubble, then that bubble had an advantage, and was placed at the top of the natural selection waiting list. Primitive reproduction can be envisioned when the bubbles burst, releasing the results of the experiment into the surrounding medium. Once enough of the 'right stuff' was released into the medium, the development of the first prokaryotes, eukaryotes, and multi-celluar organisms could be achieved. This theory is expanded upon in the book, *The Cell: Evolution of the First Organism* by Joseph Panno Ph.D.

### Origin of eukaryotic cells

The eukaryotic cell seems to have evolved from a symbiotic community of prokaryotic cells. It is almost certain that DNA-bearing organelles like the mitochondria and the chloroplasts are what remains of ancient symbiotic oxygen-breathing bacteria and cyanobacteria, respectively, where the rest of the cell seems to be derived from an ancestral archaean prokaryote cell – a theory termed the endosymbiotic theory.

There is still considerable debate on if organelles like the hydrogenosome predated the origin of mitochondria, or viceversa: see the hydrogen hypothesis for the origin of eukaryotic cells.

## History

- 1632–1723: Antony van Leeuwenhoek teaches himself to grind lenses, builds a microscope and draws protozoa, such as *Vorticella* from rain water, and bacteria from his own mouth.
- 1665: Robert Hooke discovers cells in cork, then in living plant tissue using an early microscope.[1]
- 1839: Theodor Schwann and Matthias Jakob Schleiden elucidate the principle that plants and animals are made of cells, concluding that cells are a common unit of structure and development, and thus founding the cell theory.
- The belief that life forms are able to occur spontaneously (*generatio spontanea*) is contradicted by Louis Pasteur (1822–1895) (although Francesco Redi had performed an experiment in 1668 that suggested the same conclusion).
- Rudolph Virchow states that cells always emerge from cell divisions (*omnis cellula ex cellula*).
- 1931: Ernst Ruska builds first transmission electron microscope (TEM) at the University of Berlin. By 1935, he has built an EM with twice the resolution of a light microscope, revealing previously-unresolvable organelles.
- 1953: Watson and Crick made their first announcement on the double-helix structure for DNA on February 28.
- 1981: Lynn Margulis published *Symbiosis in Cell Evolution* detailing the endosymbiotic theory.

## See also

- A549 cell
- Cariology is the study of the cell nucleus.
- Cell culture
- Cell types
- Cytorrhysis

- Cytotoxicity
- Plant cell
- Plasmolysis
- Stem cell
- Syncytium

# External links

- The cell like a city (http://www.biopic.co.uk/cellcity/cell.htm).
- Cells Alive! (http://www.cellsalive.com/)
- Journal of Cell Biology (http://www.jcb.org/)
- A simplified version of this article (http://simple.wikipedia.org/wiki/Cell)
- A comparison of the generational and exponential growth of cell populations (http://members.optusnet.com.au/exponentialist/Cells.htm)
- High-resolution images of brain cells (http://brainmaps.org/index.php?q=cell)
- Cell Biology for school and university with graphics (http://www.zytologie-online.net/)

Wikimedia Commons has media related to:
***Cell (biology)***

Wikibooks has more about this subject:
***Cell Biology***

## Online textbooks

- *Molecular Biology of the Cell (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=books&doptcmdl=GenBookHL&term=cell+biology+AND+mboc4%5Bbook%5D+AND+373693%5Buid%5D&rid=mboc4)* fourth edition, edited by Bruce Alberts (2002) published by Garland Science.
- *Molecular Cell Biology (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=books&doptcmdl=GenBookHL&term=cell+biology+AND+mcb%5Bbook%5D+AND+105032%5Buid%5D&rid=mcb.chapter.145)* fourth edition, edited by Harvey Lodish (2000) published by W. H. Freeman and Company.
- *The Cell - A Molecular Approach (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=books&doptcmdl=GenBookHL&term=cell+biology+AND+cooper%5Bbook%5D+AND+165077%5Buid%5D&rid=cooper.chapter.89)* second edition, by Geoffrey M. Cooper (2000) published by Sinauer Associates.

# References

1. ^ "*... I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular [..] these pores, or cells, [..] were indeed the first microscopical pores I ever saw, and perhaps, that were ever seen, for I had not met with any Writer or Person, that had made any mention of them before this. . .*" – Hooke describing his observations on a thin slice of cork. Robert Hooke (http://www.ucmp.berkeley.edu/history/hooke.html)

- *This article contains material from the* Science Primer (http://www.ncbi.nlm.nih.gov/About/Primer) *published by the NCBI, which, as a US government publication, is in the public domain at http://www.ncbi.nlm.nih.gov/About/disclaimer.html.*

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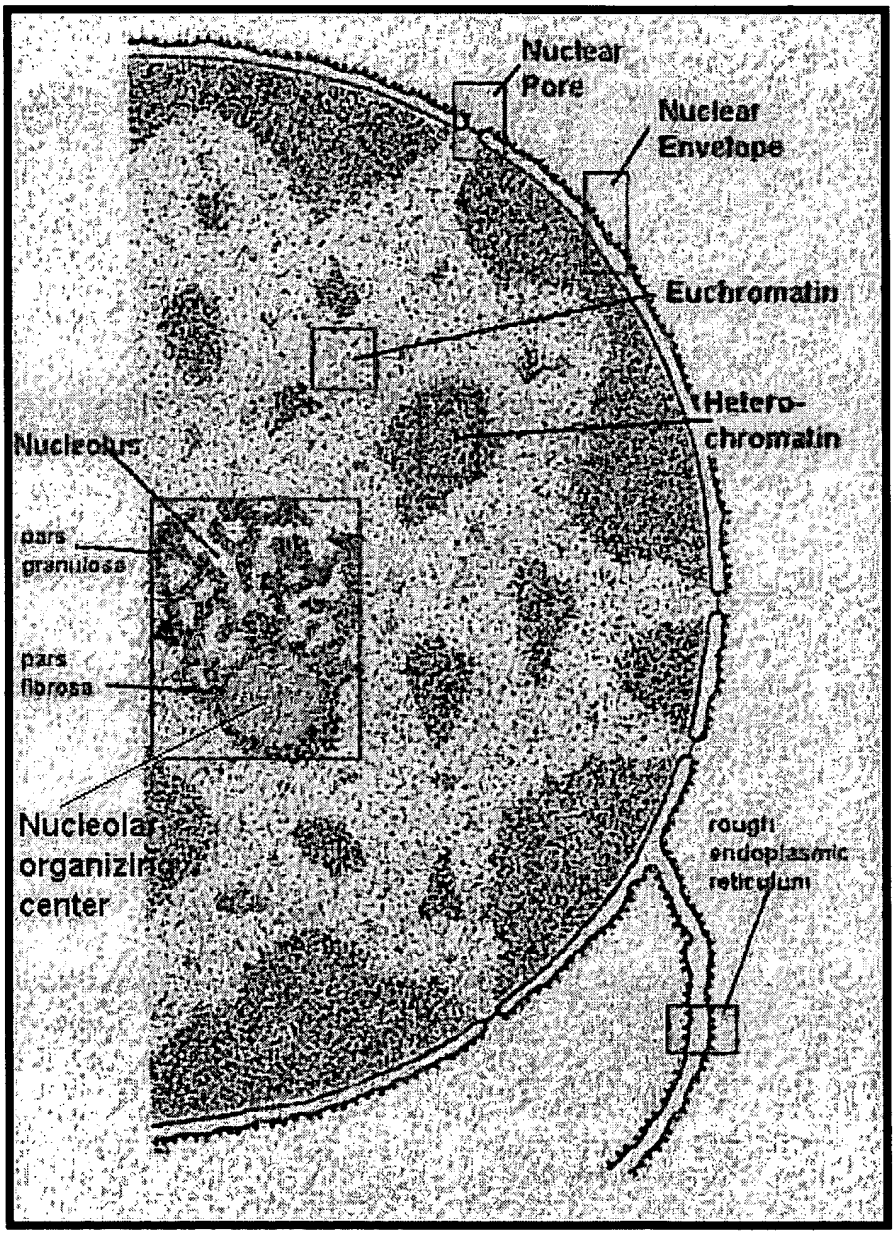
Categories: Articles to be merged | Biology | Cell biology

# EXHIBIT J

**publication entitled, "The Cell Nucleus"**
**University of Texas Medical Branch, Cell Biology Graduate Program**

| UTMB | University of Texas Medical Branch |
|---|---|
| | Cell Biology Graduate Program |

# The Cell Nucleus




## Structure/function correlations

The cell nucleus is a remarkable organelle because it forms the package for our genes and their controlling factors. It functions to:

- Store genes on chromosomes
- Organize genes into chromosomes to allow cell division.
- Transport regulatory factors & gene products via nuclear pores
- Produce messages ( messenger Ribonucleic acid or mRNA) that code for proteins
- Produce ribosomes in the nucleolus
- Organize the uncoiling of DNA to replicate key genes

**For updated information please consult: http://www.cytochemistry.net/Cell-biology/Nucleus.htm**
**For other Cell Biology topics, consult: http://www.cell-biology.org/**

The purpose of this presentation will be to show how the nucleus is structured to perform these functions. As you study the presentation, consult your text, *Alberts et al., Molecular Biology of the Cell, Garland Pub., N.Y., Third Edition, 1994 pp 335-336 for more information and photos.*

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***Last updated: 12/05/03***

***URL Address: http://cellbio.utmb.edu/microanatomy/***

# EXHIBIT K

**Patent No. 6,844,312, issued on January 18, 2005 to Weiss et al.**

Osiris THERAPEUTICS, INC.



**Media Contac**

Lisa Rodemann
Director of Hum
410-522-5005 :

## Osiris Reaches Safety Milestone in Stem Cell Clinical Trial for Cardiac Patients

Baltimore, MD, November 04, 2005 - Osiris Therapeutics, Inc. announced today that its multi-center, human clinical stem cell trial for the treatment of patients suffering from heart attacks has successfully passed the first safety milestone of the trial. Additionally, the novel stem cell therapy was cleared by the presiding independent safety board to begin enrolling patients at higher doses of drug. Osiris is developing Provacel as part of a comprehensive strategic alliance with Boston Scientific Corporation (NYSE: BSX) for development and commercialization of Osiris' mesenchymal stem cell technology in the cardiac field.

"Each step brings us closer to providing a novel stem cell therapy to treat heart attack patients who are in need," said Cardiologist Nabil Dib, M.D., Chief of Cardiovascular Research at the Arizona Heart Institute. Dr. Dib is one of the investigators evaluating the stem cell drug in patients who have recently had their first heart attack. Congestive heart failure is a common outcome in heart attack patients and is the number one cause of disability in the United States.

Enrollment in this Phase I study began in March. The trial is being conducted in accordance with U.S. Food and Drug Administration guidelines, and is designed to evaluate safety and investigate the therapeutic benefits of treatment with stem cells obtained from healthy unrelated adult donors. In accordance with the design of the trial, an independent monitoring board evaluated the safety data from the first group of patients treated with the drug as compared to those receiving placebo. Based upon predetermined criteria for the severity and number of treatment related adverse events, the board unanimously recommended that the study proceed and that a higher dose be evaluated. The Data Safety Monitoring Board is made up of independent physicians, a statistician, an ethicist and a clinical trial specialist.

Our commitment in this new frontier of stem cell therapy is first and foremost patient safety, said C. Randal Mills, Ph.D., President and CEO of Osiris Therapeutics. "This is significant

for all of our clinical programs since our stem cells products represent a true platform technology."

"We are very pleased that Osiris has reached this important safety milestone," said Dr. Jim Barry, Boston Scientific Vice President for Corporate Research and Advanced Technology Development. "This successful step reinforces our excitement about the potential of Osiris' stem cell therapy to provide a new and revolutionary treatment option for heart attack patients."

Provacel is a formulation of adult stem cells designed to repair damaged tissue. A unique benefit of the product is that it is given to patients through a standard IV line. The delivered cells are expected to respond to the body's own signals and migrate to the area of injury.

In addition to the cardiac clinical trial, Osiris is currently enrolling patients in three other stem cell studies. The company has two ongoing Phase II clinical trials with Prochymal to treat graft versus host disease, a life threatening disease affecting leukemia patients and others who have received bone marrow transplants. Osiris also has an ongoing Phase I/II clinical trial with Chondrogen for the treatment of meniscal injuries in the knee.

Osiris Therapeutics, Inc. is the leader in adult stem cell therapy. The stem cells produced by Osiris are obtained from adult volunteer donors, avoiding the technical problems and controversy surrounding other stem cell technologies. Using proprietary methods, these cells are grown in culture to very high numbers, allowing a single donor's cells to treat thousands of patients. These cells can be used in patients unrelated to the donor, without rejection, eliminating the need for donor matching and recipient immune suppression. Once transplanted, the cells promote healing of damaged or diseased tissues. The Company's current focus includes the use of adult stem cells to improve outcomes in bone marrow recipients being treated for leukemia, to repair damage following a heart attack or congestive heart failure, and to prevent and treat arthritis.

For additional information, please contact Lisa Rodemann at 410.522.5005, extension 610.
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